was invented among the arthropods after the invention of the exoskeleton."
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# Fibroblasts as Efficient Antigen-Presenting Cells in Lymphoid Organs

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**Only so-called "professional" antigen-presenting cells (APCs) of hematopoietic origin are believed capable of inducing T lymphocyte responses. However, fibroblasts transfected with viral proteins directly induced antiviral cytotoxic T lymphocyte responses in vivo, without involvement of host APCs. Fibroblasts induced T cells only in the milieu of lymphoid organs. Thus, antigen localization affects self-nonself discrimination and cell-based vaccine strategies.**

Efficient T cell activation requires two signals: "signal-1," received through the T cell receptor after engaging antigenic peptide on class I major histocompatibility complex (MHC) molecules, and "signal-2," a costimulatory signal. Originally proposed for B cells (*1*), this two-signal model is now regarded as a key mechanism in self-nonself discrimination for all lymphocyte subpopulations (*2*). Cells processing foreign antigens—professional APCs such as macrophages, dendritic cells, Langerhans cells, and B cells—would be the only cells that could provide costimulatory signals and therefore induce immune responses. Because all other cells of the body cannot provide costimulation, tissue-specific self antigens would not induce T cell responses, and autoimmunity would be avoided.

We now show that cells other than professional APCs can directly induce T cells. Mice injected with fibroblasts expressing viral proteins developed strong antiviral cytotoxic T lymphocyte (CTL) responses, without any involvement of professional APCs. Instead, class I MHC molecules on the fibroblasts were required for T cell induction. This result was unexpected, because the current model of T cell activation would predict that fibroblasts might anergize T cells, and CTL responses generated in these mice should have occurred only after antigen uptake and presentation of the fibroblast antigens by professional APCs (*2*). However, the additional finding that fibroblasts induced T cell responses only when they reached lymphoid organs may explain the discrepancy with other studies that showed that fibroblasts or tumor cells were unable to present antigen in an immunogenic manner (*3–5*).

The fibrosarcoma cell line MC57, which is derived from C57BL/6 (*H-2*$^b$ ) mice, was transfected with the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) to create a prototype of a nonprofessional APC (MC-GP) carrying a defined antigen (*6*). GP contains the dominant class I MHC epitope for CTL against LCMV in *H-2*$^b$ mice [amino acids 33 to 41 presented on D$^b$ and K$^b$ (*7*)]. MC-GP cells expressed GP on the cell surface (Fig. 1A) and were susceptible to LCMV-specific CTL lysis (Fig. 1E). D$^b$ expression was comparable to that on a lymphoma cell line (EL-4) (Fig. 1B). ICAM-1 and LFA-1, known to facilitate costimulation (*8*), were not detectable on MC-GP (Fig. 1C). MC-GP was also negative for expression of B7 molecules when stained with CTLA-4Ig (Fig. 1D) (*9*). A functional assay eliminated the possibility that MC-GP cells produced significant amounts of costimulatory cytokines. Unless exogenous cytokines were added, MC-GP cells could not restimulate memory CD8$^+$ T cells specific for LCMV in vitro, which have a minimal costimulatory requirement compared to naïve T cells (*10*) (Fig. 1E). Thus, MC-GP cells did not express costimulatory molecules and did not produce functionally significant amounts of costimulatory cytokines.

In initial studies we examined whether MC-GP cells could induce CD8$^+$ CTL responses against GP in vivo. Syngeneic C57BL/6 (*H-2*$^b$ ) mice were immunized intraperitoneally with MC-GP cells, and subsequent CTL responses against GP were assessed by means of in vivo and in vitro assays (Table 1, experiments A to C) (*11*). Mice immunized with MC-GP cells developed CTL-mediated protective immunity against lethal LCMV-induced choriomeningitis and were resistant to challenge infection with a vaccinia LCMV-GP recombinant virus, and LCMV-specific cytotoxicity could be restimulated in vitro. CTL-dependent in vivo protection was also induced in CD4$^+$ T cell–depleted mice (*12*) (Table 1, experiment C), suggesting that T help was not limiting. In summary, immunization with MC-GP cells induced strong GP-specific CTL responses.

We did the following experiments to examine the involvement of host APCs in CTL induction by MC-GP:

1) If host APCs were involved in the

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generation of GP-specific CTLs, this would occur by phagocytosis of MC-GP cellular debris containing GP. We therefore tested whether it was possible to induce GP-specific CTLs by injecting homogenized and sonicated MC-GP cells into C57BL/6 ($H\text{-}2^b$) mice. Injections were done either once or daily for 6 days, thereby simulating the situation after injection of live cells, where cellular debris may be continuously produced by tumor growth (Table 1, experiment B). However, these mice were not protected against infection with LCMV or vaccinia LCMV-GP recombinant virus (vacc-GP), and no GP-specific cytotoxicity could be detected in vitro. Thus, intact MC-GP cells were required for GP-specific CTL induction.

2) Cross-priming by host MHC is commonly used to show the involvement of host APCs (*13*). If the GP in the $H\text{-}2^b$ – fibroblast MC-GP cells could induce $H\text{-}2^s$ – restricted CTLs when injected into $H\text{-}2^s$ mice, such cross-priming would indicate that GP had been processed by $H\text{-}2^s$–host professional APCs. $H\text{-}2^s$ mice were chosen for these cross-priming experiments because their CTL response to GP is similar to that shown by $H\text{-}2^b$ mice (*7*). However, B10.S ($H\text{-}2^s$) mice immunized with MC-GP cells were not resistant to infection with LCMV or vacc-GP (Table 1, experiment D). Similarly, no cross-priming was detectable in $(H\text{-}2^{bxs})F_1$ mice immunized with MC-GP cells, demonstrating that lack of cross-priming was not caused by rejection of MC-GP cells (*14*). Conversely, MC-GP cells induced GP-specific, $D^b$-restricted CTLs even in partially allogeneic hosts, such as B10.A(4R) ($H\text{-}2K^kD^b$) mice (Table 1, experiment D).

3) A direct role for class I molecules on fibroblasts in T cell priming was then shown (Table 1, experiments E to G). The fibroblast cell line L929 ($H\text{-}2^k$ ) was transfected with the nucleoprotein (NP) of LCMV alone (L929-NP) or with both NP and the $L^d$ MHC molecules (L929-NP.$L^d$) (*14*). $L^d$ presents the dominant NP class I–binding peptide of the CTL response to LCMV in $H\text{-}2^d$ mice (amino acids 118 to 127) (*7*). In vitro, L929-NP.$L^d$ cells were lysed by $H\text{-}2^d$ – restricted CTLs (Fig. 2A), and lysis was abrogated by monoclonal antibodies to $L^d$ (Fig. 2B). In $H\text{-}2^d$ mice immunized with either L929-NP or L929-NP.$L^d$, only mice receiving L929-NP.$L^d$ showed T cell responses, as assessed by antiviral protection (Table 1, experiment E) and $^{51}Cr$-release assays (Fig. 2C).

These experiments demonstrated that the $L^d$ molecule was required on the fibroblasts to induce CTL responses, proving that fibroblasts can directly induce T cell responses. We investigated whether NP could be processed and presented by host APCs in $(H\text{-}2^d) \times (H\text{-}2^k)F_1$ mice. Again, in these mice there was no protection against challenge with LCMV or vacc-NP, unless the $L^d$ molecule was present (Table 1, experiment F). Thus, even in this histocompatible situation, host APCs did not efficiently reprocess NP (*16*). Also, immunization of $H\text{-}2^d$ mice with disrupted L929-NP or disrupted L929-NP.$L^d$ did not confer antiviral protection (Table 1, experiment E). Cross-priming was similarly undetectable in other mouse strains such as ICR ($H\text{-}2^q$) mice, which are also CTL high responders to NP (Table 1, experiment G) (*7*). Thus, fibroblasts directly induced CTLs. Similarly to mice immunized with MC-GP, there was no detectable involvement of professional host APCs in mice immunized with L929 cells.

The role of antigen localization was assessed by injection of various doses of MC-GP, either subcutaneously or intraperitoneally. Intraperitoneal injections required only 1% of the cells injected subcutaneously to induce a CTL response (Table 2, experiments A and B), and only after this route of immunization could MC-GP cells be recovered from the spleen. This suggested that the higher priming efficiency observed with intraperitoneal injections might be explained by the higher probability that fibroblasts could reach lymphoid organs. Fibroblasts

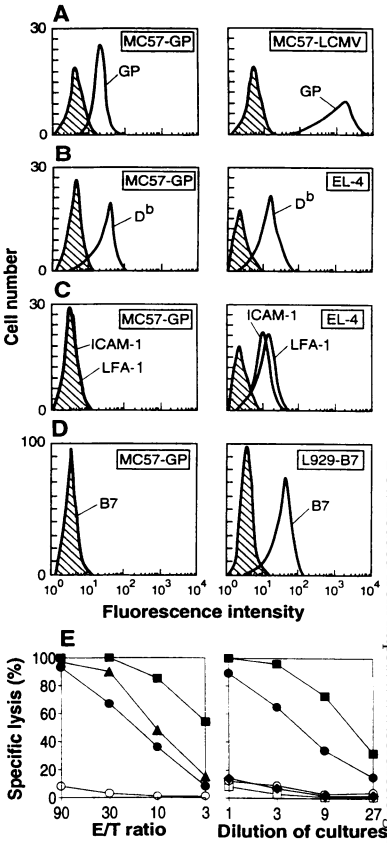


**Fig. 1.** Surface expression of GP, $D^b$, and costimulatory molecules on MC-GP. (**A**) LCMV-GP. Open lines: MC-GP (left) and LCMV-infected MC57 (right) stained with polyclonal rabbit anti-LCMV (*25*) and goat anti-rabbit IgG–FITC. Shaded lines: MC-neo (left) and MC57 (right), stained with the same two antibodies. (**B**) $D^b$. Open lines: biotinylated anti-$D^b$ and streptavidin-phycoerythrin (PE) (*25*). Shaded lines: streptavidin-PE only. (**C**) ICAM-1 and LFA-1. Open lines: biotinylated anti–ICAM-1 or anti–LFA-1 and streptavidin-PE (*25*). Shaded lines: streptavidin-PE only. (**D**) B7. Open lines: staining with CTLA-4Ig and anti-human IgG–FITC. Shaded lines: human control serum and anti-human IgG–FITC (*25*). L929 cells transfected with B7 served as a positive control. (**E**) (Left): MC-GP as a target cell in vitro. GP-specific effector CTLs were generated in C57BL/6 ($H\text{-}2^b$) mice by intravenous LCMV infection on day −9 (*26*). Effector cells were incubated for 5 hours with $^{51}Cr$-labeled MC57 infected with LCMV (squares), MC57 labeled with GP peptides (triangles), MC-GP (closed circles), and MC-neo (open circles). Spontaneous $^{51}Cr$-release was <20% for all targets shown. (Right) MC-GP as a stimulator cell in vitro. LCMV-specific memory CTLs from C57BL/6 mice were restimulated in vitro with LCMV-infected peritoneal macrophages (closed squares), noninfected peritoneal macrophages (open squares), or MC-GP (diamonds) in the absence of concanavalin A (ConA) supernatant. MC-GP cells (closed circles) and MC-neo cells (open circles) in the presence of ConA supernatant (10% v/v). All effectors were tested on MC57 target cells infected with LCMV (*26*). Specific lysis on uninfected MC57 control targets was <22% for all effectors shown. Spontaneous $^{51}Cr$-release was <14% for all targets.

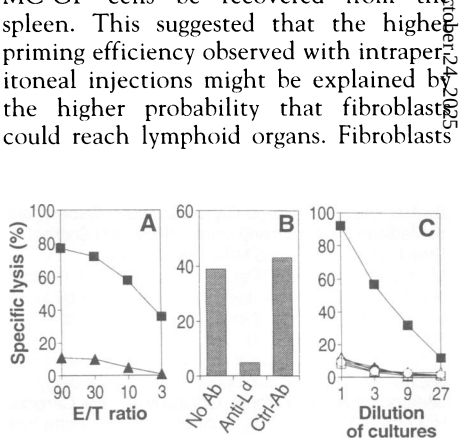


**Fig. 2.** CTL induction requires the presence of syngeneic class I MHC molecules on the fibroblast. (**A**) Comparison of L929-NP (triangles) and L929-NP.$L^d$ (squares) as a target for $H\text{-}2^d$–restricted LCMV-specific CTLs that were generated in BALB/c ($H\text{-}2^d$ ) mice immunized with LCMV on day −8 (*26*). (**B**) Lysis of L929-NP.$L^d$ is blocked by anti-$L^d$ monoclonal antibodies. $H\text{-}2^d$–restricted effector CTLs from LCMV-infected BALB/c mice were incubated at an E/T ratio of 100 (*23*). (**C**) Comparison of L929-NP and L929-NP.$L^d$ for CTL induction in BALB/c ($H\text{-}2^d$) mice. Immunization with L929-NP (triangles), L929-NP.$L^d$ (squares), or nontransfected L929 fibroblasts (circles). Ten days after immunization, spleen cells were restimulated in vitro with LCMV-infected peritoneal macrophages for 5 days and then tested on $^{51}Cr$-labeled LCMV-infected (closed symbols) or noninfected (open symbols) D2 ($H\text{-}2^d$) fibroblasts (*26*). Spontaneous release was <16% for both targets.

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**Table 1.** Induction of CTL responses with MC-GP (*H-2$^b$*) cells and transfected L929 (*H-2$^k$*) cells.

| Exp. | Immunization | | | CTL induction | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cells* | Dose (no. of cells) | Recipient mice† | Mouse survival‡ | Vacc-GP or vacc-NP§ challenge (log PFU) | LCMV-specific cytotoxicity after restimulation in vitro (lysis %) | | | |
| | | | | | | 1/1‖ | 1/3 | 1/9 | 1/27 |
| A | MC-GP | $5\times10^6$ | *H-2$^b$* | 6/6 | <0.7 | 98 | 62 | 24 | 13 |
| | MC-GP–irradiated | $2\times10^7$ | *H-2$^b$* | 3/3 | <0.7 | 100 | 88 | 70 | 32 |
| | MC-neo | $2\times10^7$ | *H-2$^b$* | 0/3 | 6.25 ± 0.49 | 16 | 5 | 3 | 3 |
| B | MC-GP–disrupted | $2\times10^7$(1×) | *H-2$^b$* | 0/3 | 6.00 ± 0.50 | | | ND | |
| | | $2\times10^7$(6×) | *H-2$^b$* | 0/3 | 5.78 ± 0.47 | 10 | 10 | 4 | 0 |
| C | MC-GP | $2\times10^7$ | *H-2$^b$* anti-CD4 | 3/3 | ND | | | ND | |
| D | MC-GP | $5\times10^7$ | *H-2$^s$* | 0/6 | 5.87 ± 0.63 | | | ND | |
| | MC-neo | $5\times10^7$ | *H-2$^s$* | 0/3 | 5.29 ± 0.78 | | | ND | |
| | MC-GP | $2\times10^7$ | *H-2 K$^k$ D$^b$* | 3/3 | ND | | | ND | |
| E | L929-NP | $1\times10^6$ | *H-2$^d$* | 0/6 | 6.20 ± 0.45 | | | ND | |
| | L929-NP–disrupted | $1\times10^7$ | *H-2$^d$* | 0/3 | 5.73 ± 0.25 | | | ND | |
| | L929-NP.L$^d$ | $1\times10^6$ | *H-2$^d$* | 6/6 | <0.7 | | | ND | |
| | L929-NP.L$^d$–disrupted | $1\times10^7$ | *H-2$^d$* | 0/3 | 5.50 ± 0.46 | | | ND | |
| F | L929-NP | $1\times10^7$ | *H-2$^{dxk}$* | 0/3 | ND | | | ND | |
| | L929-NP.L$^d$ | $1\times10^7$ | *H-2$^{dxk}$* | 3/3 | ND | | | ND | |
| G | L929-NP | $1\times10^7$ | *H-2$^q$* | 0/3 | 6.95 ± 0.61 | | | ND | |
| | L929 | $1\times10^7$ | *H-2$^q$* | 0/3 | 6.41 ± 0.16 | | | ND | |

*Untreated, gamma-irradiated (3000 rad) or disrupted cells (homogenized and sonicated) were injected in saline in a volume of 400 μl. †C57BL/6 (*H-2$^b$*), B10.S (*H-2$^s$*), B10.A (4R) (*H-2 K$^k$ IA$^k$ D$^b$*), BALB/c (*H-2$^d$*), (BALB/c × CBA)$F_1$ (*H-2$^{dxk}$*), or ICR (*H-2$^q$*) were used as recipient mice. In exp. C, C57BL/6 mice were depleted of CD4$^+$ T cells in vivo before immunization by the use of a monoclonal antibody (*12*). ‡Mice were intracerebrally challenged with LCMV (isolate Armstrong, 30 PFU in 30 μl). All mice that were not protected died within 9 days; surviving mice were observed until day 30. §Mice immunized with MC-GP (exp. A to D) were challenged intraperitoneally with vacc-GP ($1\times10^7$ PFU in 300 μl), and mice immunized with NP-transfected L929 (experiments E to G) were challenged with vacc-NP ($1\times10^7$ PFU in 300 μl). After 5 days vaccinia virus titers were determined in both ovaries. Titers are given as mean log PFU ± SD per both ovaries of three individual mice. ‖Spleen cells were restimulated in vitro with LCMV-infected, thioglycollate-stimulated peritoneal macrophages. After 5 days cultures were harvested and 1:3 dilutions were tested for LCMV-specific cytotoxicity on LCMV-infected target cells (*26*). Specific $^{51}$Cr-release was less than 14% on uninfected targets for all effectors shown.

were found to be very potent APCs upon direct injection into the spleen, where as few as 500 irradiated MC-GP cells were sufficient to induce GP-specific CTLs (Table 2, experiment C).

We now show that transfected fibroblasts lacking costimulatory function but expressing a class I–associated virus peptide could directly and efficiently induce CTL responses if they reached the cytokine-rich lymphoid environment. There was no apparent need for a link of antigen and costimulatory signals on the same cell. This confirms results of earlier studies suggesting that fibroblasts could directly induce allogeneic T cells (*17*). We extend these observations to defined nominal antigens and demonstrate that T cell activation by subcutaneous or intraperitoneal injection of fibroblasts appears to be the result of a few fibroblasts draining to lymphoid organs.

**Table 2.** Role of site of injection in determining immunogenicity of fibroblasts.

| Number of cells | Mouse survival§ | Recovery of MC-GP from spleen‖ | Specific cytotoxicity (%)¶ | | | |
|---|---|---|---|---|---|---|
| | | | 1/1 | 1/3 | 1/9 | 1/27 |
| | | *A. Subcutaneous injection of MC-GP** | | | | |
| $1\times10^7$ | 2/3 | ND | | | | |
| $1\times10^6$ | 0/3 | – | | | | |
| $1\times10^5$ | 0/3 | ND | | | | |
| $1\times10^4$ | 0/3 | – | | | | |
| | | *B. Intraperitoneal injection of MC-GP†* | | | | |
| $1\times10^7$ | 3/3 | ND | | | | |
| $1\times10^6$ | 3/3 | + | | | | |
| $1\times10^5$ | 3/3 | ND | | | | |
| $1\times10^4$ | 0/3 | – | | | | |
| | | *C. Intrasplenic injection of MC-GP‡≠* | | | | |
| $1\times10^4$ | | | 92 | 53 | 21 | 5 |
| 500 | | | 69 | 41 | 25 | 3 |

*Indicated number of MC-GP cells injected subcutaneously on the back (50 μl in saline). †Indicated number of MC-GP cells injected intraperitoneally (50 μl in saline). ‡Indicated number of MC-GP cells injected directly into the spleen (20 μl in saline). §Intracerebral injection with LCMV isolate Armstrong (30 PFU in 30 μl). All mice that were not protected died within 9 days; surviving mice were observed until day 30. ‖Ten days after injection of MC-GP cells, single spleen cell cultures ($3\times10^6$ spleen cells) were grown in the presence of G418 selecting for the drug-resistant MC-GP cells. In the wells designated positive (+), fibroblasts were still growing after 10 days. ¶LCMV-specific lysis on EL-4 (*H-2$^b$*) target cells labeled with GP peptide after in vitro restimulation with LCMV-infected peritoneal macrophages (*25*). Spontaneous release was less than 20%. Specific lysis on EL-4 control cells without peptide was less than 15%. ≠MC-neo cells ($1\times10^4$) injected into the spleen gave 2% specific activity at 1/3 to 1/27 E/T and 9% specific activity at 1/1 E/T.

These results impinge on a key question: What defines the immunogenicity of an antigen? Costimulatory molecules or cytokines, such as B7 or interleukin-2 (IL-2) are regarded as most important (*2–5*, *9*). The fibroblasts studied here did not have such costimulatory properties and a lymphoid environment was sufficient for CTL induction. Thus, immunogenicity was determined by antigen localization.

Previous in vitro and in vivo data suggested an exclusive role for professional APCs in inducing naïve T cells. In vitro, costimulatory signals from professional APCs facilitate T cell responses (*18*). In vivo, the requirement for costimulation was demonstrated with tumor cells displaying specific antigens that did not induce T cells, unless transfected with costimulatory molecules or cytokines (*3*), and similar experiments with transgenic mice showed that antigens exclusively expressed in peripheral solid tissues were not immunogenic (*4*). The requirement for professional APCs was classically demonstrated by Lafferty, who showed that histoincompatible organ grafts placed under the kidney capsule were

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not rejected when depleted of professional APCs (5). Taken together, the above independent observations—that in vivo, both costimulation and professional APCs were required for T cell induction—led to the conclusion that professional APCs were required because they provided costimulation.

However, in vivo the role of professional APCs has not been dissected into its two important components: the potential to provide costimulation, as compared with the ability to migrate with acquired antigen into lymphoid organs. The above-mentioned studies did not discriminate between these two aspects, because professional APCs were the only cells able to migrate into lymphoid organs. That this migration was an essential property for immunogenic APC function had already been suggested by earlier studies (*19*), where blocking of lymphoid vessels draining a skin flap prevented T cell responses to antigens on the flap. Our present data extend these observations. We show that fibroblasts can function as immunogenic APCs in the milieu of the lymphoid organs. Thus, transport of antigen into lymphoid organs is not only necessary but is sufficient for immunogenic APC function, whereas costimulatory properties do not have to be directly linked to this cell.

We propose that self-nonself discrimination is not based on the costimulatory capacity exclusive to professional APCs (*1*, *2*), but rather on a simple geographical model: Antigens outside of lymphoid organs are ignored by T cells, whereas antigens newly transported into lymphoid organs provoke a response. Those antigens that are generally present in lymphoid organs cause deletion of T cells in the thymus or periphery (*20*). Because antigens are usually transported into lymphoid organs by professional APCs, the latter are normally crucial for T cell induction.

The immunogenicity of the lymphoid environment may be explained by several factors, such as high T cell traffic, highly organized organ structure, and sequential exposure to antigen. The abundance of costimulatory cytokines in lymphoid organs certainly plays an important role, as also suggested by the finding that MC-GP stimulated T cells in vitro only when exogenous cytokines were added.

In contrast to the involvement of host APCs in mice carrying melanomas and lymphomas (*13*), we observed no processing of tumor-specific antigens by host APCs. These findings are not necessarily inconsistent. Both fibroblast tumors used here were directly immunogenic, so the cells were probably cleared before large amounts of tumor-specific antigen accumulated. Conversely, host APC involvement could be favored by the fact that spontaneous lymphomas probably have been selected for the reduction or absence of antigenicity and direct immunogenicity, because otherwise outgrowth in a lymphoid environment could not occur. Melanomas also may be exceptional, in that they escape immune surveillance by losing their direct immunogenicity (for example, by loss of MHC class I) (*21*).

Our observations may explain some long-standing paradoxes of tumor immunology. A phenomenon called "sneaking through" (*22*)—that is, small numbers of tumor cells may grow after subcutaneous injection, whereas large inocula often do not—may simply reflect the fact that, after injection of small numbers of cells, the cells reach lymph nodes only after a large tumor burden is already established. That immune responses are finally induced may explain the phenomenon of "concomitant immunity" (*23*): Some mice carrying large tumors are resistant to tumor challenge at distant sites. Another general observation, that tumors can be transplanted as small solid pieces but usually not as cell suspensions, may be explained by cells in suspension being more likely to reach lymph nodes and induce an early protective immune response.

Finally, our findings may be important for tumor vaccination strategies. Current strategies aim at the artificial creation of a cytokine-rich immunogenic environment by transfection of tumor cells with costimulatory molecules or cytokines, such as B7, IL-2, IL-4, IL-6, IL-7, IL-12, G-CSF, GM-CSF, or interferon γ (*3*, *24*). We invert this concept and show that tumors can be made immunogenic simply by delivery into lymphoid organs that are naturally rich in cytokines.

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74,29 (1983)]. Monoclonal antibody to $L^d$ (28-14-8.S) was provided by M. Lohoff (University of Erlangen, Erlangen, Germany). As an irrelevant (control) antibody for anti-$L^d$, a rat antibody to human glycophorin IgG was used (YTH 89.1).

26. Primary cytotoxicity and cytotoxicity after secondary in vitro restimulation were assessed as described [R. M. Zinkernagel *et al.*, *J. Exp. Med.* **162**,2125 (1985)]. Target cells were infected with either LCMV [0.1 plaque-forming units (PFU) per cell] 48 hours before the assay, or with vacc-GP (5 PFU per cell) 2 hours before the assay, or labeled with the $K^b$-binding LCMV-GP peptide aa33-41 (50 mM for 2 hours) [H. P. Pircher *et al.*, *Nature* **346**, 629 (1990)].

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# Mutations in Fas Associated with Human Lymphoproliferative Syndrome and Autoimmunity

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**Fas (also known as Apo1 and CD95) is a cell surface receptor involved in apoptotic cell death. Fas expression and function were analyzed in three children (including two siblings) with a lymphoproliferative syndrome, two of whom also had autoimmune disorders. A large deletion in the gene encoding Fas and no detectable cell surface expression characterized the most affected patient. Clinical manifestations in the two related patients were less severe: Fas-mediated apoptosis was impaired and a deletion within the intracytoplasmic domain was detected. These findings illustrate the crucial regulatory role of Fas and may provide a molecular basis for some autoimmune diseases in humans.**

The life and death of lymphocytes are tightly controlled by membrane receptors that activate either proliferative or apoptotic processes (*1*). The Fas antigen has been identified as a key cell surface receptor involved in apoptotic cell death (*2*). Fas is a cell surface protein of 48 kD that plays a major role in induction of apoptosis in lymphoid cells. Mutations in the Fas-encoding gene are responsible for the lymphoproliferative disorder and associated lupuslike syndrome in *lpr* and $lpr^{cg}$ mice (3). Defects in Fas-induced apoptosis may lead to incomplete elimination of peripheral autoreactive cells in these mice (4).

We studied three patients (two siblings and an unrelated child) with clinical and immunological features reminiscent of those seen in *lpr* mice: a lymphoproliferative syndrome, which, in one of the siblings, was associated with autoimmune hemolytic anemia, neutropenia, and thrombocytopenia (patient 3). A large proportion of peripheral blood (Table 1) and splenic T lymphocytes expressed αβ T cell receptors (TCR) but expressed neither CD4 nor CD8 molecules. These double-negative (DN) T cells, as well as the single-positive (SP) T cells showed normal expression of the TCR-CD3 complex. Analysis of TCR-Vβ utilization in DN T cells from patient 3 showed this population to be oligoclonal. The expression of Fas on activated T cells was markedly reduced in patient 1 but was comparable in patients 2 and 3 and in healthy controls (Fig. 1A). In contrast, Fas-mediated apoptosis, determined by means of hypodiploid nuclei quantification (5), was defective in T cells (Fig. 1B) and in Epstein-Barr virus–transformed B cells from all three patients: A monoclonal antibody (mAb) to Fas caused apoptosis in 65% of control cells but no detectable apoptosis in patient 1 and only 20% apoptosis in cells from patients 2 and 3. The same results were achieved with the use of the TUNEL method (6). Cells from the father but not the mother of patients 2 and 3 showed a mild reduction in Fas-mediated apoptosis (Fig. 1B), as shown with the use of both hypodiploid nucleic quantification and TUNEL methods. Staphylococcal enterotoxin B–induced apoptosis in patient 1 and antibody to CD3-induced apoptosis in patients 2 and 3 were reduced. This shows that the Fas–Fas ligand interaction does not operate in these Fas-deficient cells.

Northern (RNA) blot analysis (Fig. 2B) showed a reduction in the transcript

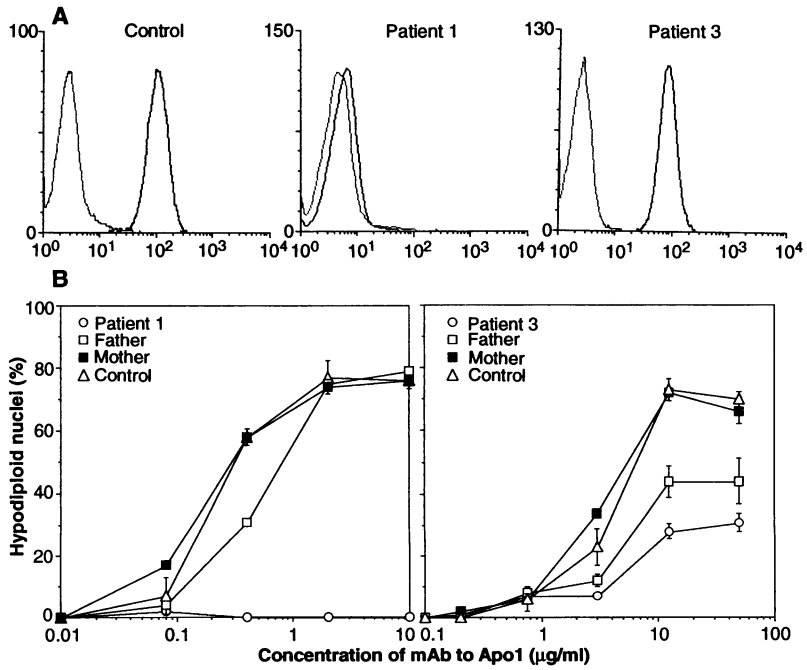


**Fig. 1.** (**A**) Defective Fas expression on activated T cells. (**B**) Defective Fas-mediated apoptosis. Shown is the percentage of hypodiploid nuclei (*13*) as a function of the concentration of mAb to Apo1 in cells from patients 1 and 3 (open circles), from fathers (open squares), from mothers (solid squares), and from two controls (open triangles). The same results were obtained for patients 2 and 3 in three separate experiments. The difference in sensitivity of the Fas-induced apoptosis assay between patients 1 and 3 is secondary to the use of different batches of mAb to Fas.

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